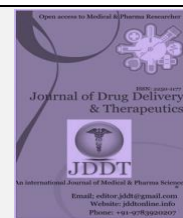
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Research Article

Formulation and Evaluation of Fixed Dose Combination Tablets of Antifungal Drugs for *Candida albicans* Resistant to Fluconazole

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ABSTRACT

Background: The emergence of *Candida albicans* strains resistant to fluconazole (FLZ) had raised interest on combining other antifungals with FLZ. In vitro and clinical studies had indicated synergistic interaction between terbinafine and FLZ against strains resistant to FLZ and improved therapeutic efficacy. **Objective:** Formulation and evaluation of fixed dose combination (FDC) tablets of terbinafine HCl (TH) and FLZ for avoidance of resistance and improved therapeutic efficacy against *C. albicans* infections. **Method:** The compatibility of TH and FLZ together and with excipients was determined by FTIR spectroscopy. A UV-visible spectroscopic Q-absorbance ratio method was developed and validated for linearity, accuracy, precision, LOD and LOQ for simultaneous estimation of TH and FLZ. The FDC tablets was prepared by wet granulation using hydroxy propyl cellulose (1%, 2%, 3%, 4% and 5%) as binder and evaluated for pre-compression and post-compression parameters. **Result and Discussion:** The TH and FLZ were compatible together and with excipients used in FDC. The linearity range was found to be 0.5-3.0 µg/ml and 80-400 µg/ml for TH and FLZ, respectively. Percent RSD was less than 2% indicating good accuracy and precision for proposed method. The hardness and disintegration time of tablets increased and friability decreased with increased binder concentration. Formulation F2 and F3 showed more than 80% drug release within 30 minutes. **Conclusion:** The TH and FLZ were compatible and can be formulated as a FDC tablet. The UV-Visible spectrophotometric method developed for simultaneous estimation was simple, accurate and sensitive.

Keywords: Terbinafine, Fluconazole, Q-absorbance, Fixed Dose Combination, *Candida albicans*

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1. INTRODUCTION

Candida albicans is the most important fungal strain accountable for most of the localized and systemic fungal infections¹. Systemic candidiasis is the leading cause of mycosis-associated mortality in the United States and is globally recognized as a cause of significant morbidity².

Immunocompromised patients, such as AIDS patients or are neutropenic due to cancer therapy, are at specific threat of evolving local *C. albicans* infections, which may become systemic^{3, 4}. Fluconazole (FLZ) is an orally active triazole agent, well established as a first-line treatment for localized and systemic *C. albicans* infections¹. In the past decades, advent of resistant *C. albicans* strains to FLZ on long term suppressive therapy has been reported in AIDS patient with oropharyngeal candidiasis^{5, 6}. An attractive therapeutic option in these circumstances is combining other antifungals with the FLZ for synergistic effect and widened spectrum against FLZ resistant fungal strains. The use of terbinafine an

orally and topically active antifungal has been recommended in combination with FLZ for the cases with FLZ resistant *C. albicans*⁷. Terbinafine is act as a fungicidal against a broad spectrum of fungi including dermatophytes, filamentous, dimorphic organisms and some yeast such as *Candida parapsilosis*⁸.

Synergistic interactions of terbinafine with azoles and other compounds against *Candida albicans* have been reported by many investigators. Ghonnoum and Elewski⁶ had reported the successful treatment of oropharyngeal candidiasis patients with a combination of FLZ and terbinafine who had also FLZ-refractory. In an *in vitro* study, Barchiesi and colleagues concluded a synergistic interaction between terbinafine and FLZ against *C. albicans* strain with reduced susceptibility to FLZ⁷. The above evidences suggested that combining the terbinafine and FLZ in fixed dose combinations (FDC) can be a better approach for the treatment of cases associated with FLZ resistant *C. albicans*. FDC are dosage forms which contain two or more active

ingredients in fixed proportions in same formulations. FDC can offer enough advantages as compared to traditional monotherapy like improved patients compliance, simple dosage schedule, greater efficacy compared to monotherapy, reduced adverse events and synergistic effect ⁹.

So, in the present research work, fixed dose combination (FDC) tablets of terbinafine hydrochloride (TH) and FLZ has been formulated by wet granulation using hydroxy propyl cellulose (HPC) as a binder and starch as disintegrating agent. During the preformulation studies the compatibility of the TH and FLZ together and with different excipients were evaluated. A UV spectrophotometric method i.e Q-analysis or Q-absorbance ratio for the simultaneous analysis of TH and FLZ was developed and validated. The ratio of absorbance at two wavelengths is constant for two different concentrations. In this method, the absorption of the two components is measured at two different wavelengths. One being the absorption at λ_{max} of one of the component and other is absorption at iso-absorptive point ¹⁰.

2. MATERIALS AND METHODS

Fluconazole and terbinafine HCl was procured from Lark Pharmaceuticals, Bhiwandi, India as gift sample. HPC, starch, magnesium stearate, talc and lactose were procured from CDH Chemicals, Mumbai.

2.1 Preformulation studies

Preformulation studies are carried out in order to determine the physicochemical properties of drug which helps in the design of a safe, efficacious and stable dosage form. The drugs (TH and FLZ) were physically characterized on the basis of color and odor. All the physical characterization were observed and compared with the monograph in official Pharmacopoeia. Melting point of TH and FLZ was determined by using digital melting point test apparatus.

2.1.1 Absorption maxima (λ_{max}) and preparation of calibration curve

The λ_{max} of TH was determined in 0.1N HCl by scanning the 3.0 $\mu\text{g}/\text{ml}$ solution in range of 400-200 nm using UV-Visible spectrophotometer (Lab India, Mumbai). The calibration curve of TH was prepared by dissolving 100 mg in small volume of 0.1N HCl and final volume was adjusted up to 100ml with the same solvent. A series of standard solution of concentrations 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 $\mu\text{g}/\text{ml}$ were prepared and absorbance was measured at 222 nm against blank using an UV-Visible spectrophotometer.

The λ_{max} of FLZ was determined in 0.1N HCl by scanning the 400 $\mu\text{g}/\text{ml}$ solution in range of 400-200 nm using UV-Visible spectrophotometer. An accurately weighed 100 mg of FLZ was dissolved in small volume of 0.1N HCl in the 100 ml volumetric flask and final volume was adjusted with the same solvent. A series of standard solutions of concentrations 80, 120, 160, 200, 240, 280, 320, 360 and 400 $\mu\text{g}/\text{ml}$ of FLZ were prepared. The measurements of absorbance were done at 260 nm against blank using an UV-Visible spectrophotometer.

2.1.2. Fourier transformed infrared (FTIR) studies of pure drugs

FTIR spectra of pure drug were observed and analyse by potassium bromide (KBr) press pellet method. Pure drug TH was uniformly mixed with dry powdered KBr in the ratio of 1:100 and mixture was then compressed into transparent disc under high pressure using special dies. The disc was placed in FTIR spectrophotometer (Shimadzu IR Affinity-1) and spectrum was recorded. Same procedure was followed for FLZ and spectrum was recorded.

2.1.3 Compatibility study of Drug-drug and drug-excipient by FTIR spectroscopy

TH and FLZ compatibility, together and with the excipients used in the formulation of tablets was determined by FTIR spectrophotometer. TH and FLZ physical mixture (250 mg:150 mg) was prepared by triturating them together in a clean and dried mortar-pestle. The one part of above prepared physical mixture and hundred part of FTIR grade KBr was mixed together properly. Then using KBr press pellet a thin and transparent pellet was prepared. The FTIR spectrum, of pure TH and FLZ, recorded by KBr disc method was compared for specific peak positions of different functional groups.

To determine the compatibility of TH and FLZ with excipients used for formulation of FDC tablets, triturate the TH, FLZ, HPC, starch, talc, magnesium stearate and lactose with ethanol. The prepared physical mixture was dried in a hot air oven to completely remove the ethanol and any traces of moisture at 60°C and passed through the BSS number 85. The FTIR spectrum of the physical mixture was recorded by KBr disc method and peak positions of important functional groups in pure drugs were compared.

2.2 Development and validation of simultaneous estimation method for TH and FLZ by UV-Visible spectrophotometer

Q-Absorbance ratio or Q-Analysis method for simultaneous estimation of TH and FLZ by UV-Visible spectrophotometer was developed and validated ¹⁰⁻¹².

2.2.1 Standard stock solutions of TH and FLZ

100 mg of TH was dissolved in small volume of 0.1N HCl in 100 ml volumetric flask. The solution was sonicated for 10 minutes and adjusted the final volume with 0.1N HCl. From standard stock solution (1000 $\mu\text{g}/\text{ml}$), by pipetting out 1ml in a 100 ml volumetric flask working stock solution (10 $\mu\text{g}/\text{ml}$) was prepared and final volume was adjusted with 0.1N HCl. Then in series of 10 ml volumetric flasks pipetting out 0.5, 1.0, 1.5, 2.0, 2.5 and 3 ml from working stock solution and volume was made up to the mark with the same solvent. For FLZ standard solution 100 mg of drug was dissolved in small volume of 0.1N HCl in a 100 ml volumetric flask. The solution was sonicated for 10 minutes and final volume adjusted. From above solution 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 and 4.0 ml were pipetted out in series of 10 ml volumetric flask and final volume was made up with 0.1N HCl.

2.2.2 Selection of wavelength of maximum absorbance (λ_{max})

For 3 $\mu\text{g}/\text{ml}$ solution of TH pipetting out 3 ml from working stock solution of drug in 10 ml volumetric flask and final volume was adjusted with 0.1N HCl. For 400 $\mu\text{g}/\text{ml}$ FLZ solution was prepared by pipetting out 4 ml from standard stock solution in 10 ml volumetric flask and final volume was adjusted up with 0.1N HCl. Both the solutions were scanned in the range of 400-200 nm against blank solution (0.1N HCl) using UV-Visible spectrophotometer.

2.2.3 Determination of iso-absorptive point

Iso-absorptive point was determined by preparing 1.5 $\mu\text{g}/\text{ml}$ solutions of TH and 200 $\mu\text{g}/\text{ml}$ of FLZ and by scanning in the range of 400-200 nm against 0.1N HCl as blank using an UV-Visible spectrophotometer. An overlain spectrum of TH and FLZ was found and iso-absorptive point was determined.

2.2.4 Determination of Linearity range

Linearity range of TH was determined by preparing different concentrations i.e. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 µg/ml from working standard solution and at 222 nm and 239 nm absorbance was taken against 0.1N HCl as a blank. Linearity curve was plotted between concentration vs. absorbance. Linearity of FLZ was determined by preparing different concentrations 160, 200, 240, 300, 360 and 400 µg/ml were prepared from standard stock solution and absorbance was measured at 222 nm and 239 nm against 0.1N HCl as a blank. Than linearity curve was plotted between concentration vs. absorbance.

2.2.5 Determination of Accuracy

To determine the accuracy of the proposed method 2.0, 2.5 and 3.0 µg/ml; and 240, 300 and 360 µg/ml solutions were prepared for TH and FLZ, respectively. These were the three different levels 80%, 100% and 120% by considering 2.5µg/ml and 300µg/ml as 100% for TH and FLZ, respectively. The recovery study was performed three times at each level.

2.2.6 Determination of Precision

2.2.6.1 Intermediate Precision (Inter-day and Intra-day precision)

The solutions of same concentrations were prepared as in the determination of accuracy. Inter-day precision both the drugs were analyzed individually daily once for three days at specified wavelengths as above. Intra-day precision was determined by analyzing TH and FLZ individually for three times in the same day at the wavelength 222 nm and 239 nm. The standard deviation and relative standard deviation were calculated.

2.2.6.2 Repeatability

In repeatability, 3.0 µg/ml and 300 µg/ml solutions were prepared for TH and FLZ, respectively. The measurement of absorbance was taken at 222 nm and 239 nm against 0.1N HCl as a blank. The experiment was done six times. The standard deviation and relative standard deviation was calculated.

2.2.6.3 Reproducibility

The absorbance was measured by two analysts and values obtained were evaluated using t-test to verify their reproducibility. 2.0 µg/ml and 300 µg/ml solutions were prepared from stock solutions separately of TH and FLZ, respectively and analyzed by one analyst (analyst 1) at 222 nm and 239 nm. Same solutions were prepared by another analyst (analyst 2) and values obtained were evaluated using t-test to verify their reproducibility.

2.2.7 Limit of Detection (LOD)

The LOD is defined as in a sample lowest concentration of a drug that can be detected, but not necessarily quantified, under the given conditions of the test. LOD was calculated from the obtained data from the linearity studies. The slope of the linearity plot was determined and calculated by formula given below.

$$\text{LOD} = 3.3(S/b)$$

Where,

S = Standard deviation of the residuals around the regression line.

b = Slope of the regression line.

2.2.8 Limit of Quantitation (LOQ)

The LOQ is defined as the lowest concentration of a drug in a sample that can be determined with acceptable accuracy and precision under the given conditions of test. Limit of quantitation can be calculated from standard deviation of the response and slope using formula given below.

$$\text{LOQ} = 10(S/b)$$

Where,

S = Standard deviation of the residuals around the regression line.

b = Slope of the regression line.

2.3 Formulation and Evaluation of FDC tablets

2.3.1 Formulation of granules

For the formulation of FDCs tablets wet granulation method was used and to prepare granules, HPC and starch were used as binder and disintegrant respectively. Talc, magnesium stearate and lactose were used as glidant, lubricant and diluents, respectively (Table No. 1). Firstly TH, FLZ, HPC, starch and lactose were passed through British Standard Sieve (BSS) 60. Then TH, FLZ, HPC, lactose and half of the starch were mixed uniformly and sufficient quantity of distilled water was added with continuous mixing to obtain wet dough mass. The wet mass was shifted through BSS 10 and dried in hot air oven at 60°C till moisture content was achieved in the range of 1.5-2%. The dried mass was then passed through the BSS 16/44. The granules which were retained on the BSS 44 were considered coarse granules and the granules which passed through BSS 44 are considered as fines. To the granules, remaining starch, talc and magnesium stearate previously passed through BSS 80 were mixed uniformly.

Table No. 1 Formulation formula for granules using HPC as binder

Sr. No.	Ingredient (mg)	Formulation Codes				
		F1	F2	F3	F4	F5
1	Terbinafine Hydrochloride	250	250	250	250	250
2	Fluconazole	150	150	150	150	150
3	Hydroxy propyl cellulose	10	15	20	25	30
4	Starch (10%w/w)	50	50	50	50	50
5	Talc (1%w/w)	5	5	5	5	5
6	Magnesium Stearate (1%w/w)	5	5	5	5	5
7	Lactose	35	30	25	20	15
Weight of tablet		500 mg	500 mg	500 mg	500 mg	500 mg

2.3.2 Evaluation of granules

2.3.2.1 Mean granule size

Mean granule size was determined using electric sieve shaker (Electrolab, Mumbai, India) in which different BSS were arranged in ascending order of sieve number (16, 22, 44 and 60). Accurately weighed 30 g of granules was placed on the top sieve and sieve shaker was operated for 20 min. The granules retained on each sieve were weighed and granules retained on each sieve were determined. The mean granule size was calculated by finding out the average size of the sieve opening, through which the granules were passed and the size of the sieve opening, on which the granules were retained. The weight retained (X) on each sieve was multiplied with average sieve opening (Y) of two successive sieves. The mean granule size was determined using formula given below¹³.

Mean granule size (μm) = ΣXY /Weight of granules

2.3.2.2 Bulk and tapped density

Bulk density was measured by taking 35ml granules in 50 ml measuring cylinder. The granules volume at initial stage was recorded in the form of bulk volume. The placement of measuring cylinder was done on tapped density tester (EDT-1020, Electro lab, India). The falling rate of drops was 250/minutes from the height of 3 mm \pm 10%. The volume of powder bed was recorded after every increase of 250 drops up to constant volume change. The final recorded volume was known as tapped volume. The BD and TD were measured as ratio of weight of the powder by bulk volume and tapped volume¹⁴.

2.3.2.3 Determination of Hausner ratio

Hausner ratio is an indirect index of ease of powder flow. Hausner ratio was calculated formula given below.

Hausner ratio = TD/BD

2.3.2.4 Determination of Carr's consolidation index

Carr's consolidation index was calculated by formula given below.

Carr's consolidation index (%) = $[(TD-BD)/TD] \times 100$

2.3.2.5 Determination of angle of repose

Angle of repose acts as important parameter to determined the flow property of granule. Funnel was placed and fixed at above of graph paper, which was placed on horizontal flat surface. The granule was carefully poured through the funnel until the apex of the conical pile just touched tip of the funnel. The radius (r) of base of pile and height (h) of the pile was determined. The angle of repose (θ) was calculated by using formula given below.

$$\theta = \tan^{-1} h/r$$

2.3.2.6 Percentage of fines

For the determination of percentage fines, a known weight of dried granules was passed through BSS 44. The weight of granules which passed through sieve number 44 was recorded and percentage fine was calculated with respect to total weight of granules.

2.3.2.7 Moisture content

Moisture content (%) of granules was determined using a digital moisture balance (MA45, Sartorius, Mumbai, India).

2.3.3 Compression of FDC tablets

The granules were compressed into 500 mg tablets on a 10 station rotary tablet compression machine (Rimek, Ahmadabad, India) using 13 mm die cavity.

2.3.4 Evaluation of FDC tablets

2.3.4.1 General Appearance

The compressed tablets were observed for color, shape, surface irregularities, cracks or any other defects.

2.3.4.2 Average weight

Twenty tablets were weighed individually and the average weight was calculated. The individual tablet weights were then compared to the average weight and percent deviation was calculated.

2.3.4.3 Thickness and Hardness

The thicknesses of randomly selected 10 tablets were determined using digital vernier caliper (Mitutoya, Japan). Hardness of tablets (n=10) for each batch was determined using Monsanto hardness tester (Cadmach, Ahmedabad, India).

2.3.4.4 Determination of Friability

Friability was determined using Roche friabilator (Electrolab). Initial weight of the 10 tablets was recorded (W1). The tablets were placed in the friabilator and rotated at 25 rpm for four minutes. After that, tablets were taken out and final weight was determined (W2). The percentage friability was determined by using formula given below.

$$\text{Friability (\%)} = [(W1-W2)/W1] \times 100$$

2.3.4.5 Disintegration time

The disintegration time for tablets (n=6) for F2, F3, F4 and F5 was determined using disintegration test apparatus (ED-2SAPO, Electrolab, India) at 37 \pm 0.5°C in 0.1N HCl.

2.3.4.6 Drug content

Drug content of formulation F2 and F3 was determined. Average weight of 10 tablets was determined and then tablets were crushed to form powder. Powder having weight equivalent to average weight was taken in 100 ml of volumetric flask and dissolved in small volume of 0.1N HCl. Final volume was adjusted with 0.1N HCl. Then the flask was shaken for 24 h using a water bath shaker and solution was filtered. After suitable dilution of filtrate, absorbance was measured at 222 nm and 239 nm for TH and FLZ, respectively using an UV-Visible spectrophotometer and drug content was calculated by Q-Analysis method.

2.3.4.6 *In vitro* drug release

In vitro drug release study of the tablets (F2 and F3) was performed using USP Type II dissolution test apparatus (LABINDIA DS 8000, India) using 900 ml 0.1N HCl as a dissolution medium maintained at 37 \pm 0.5°C. The paddles were rotated at speed of 75 rpm. At regular intervals of time for 1 hour, 10 ml sample was withdrawn and replaced with equal volume of 0.1N HCl to maintain the sink condition. Samples were filtered through whatmann filter (0.42 μm) paper and after appropriate dilutions absorbance was recorded at 222 nm and 239 nm for TH and FLZ, respectively using an UV-Visible spectrophotometer. The T_{80%} (Time to release 80% of drug) was calculated from percent cumulative drug release vs. time plots.

3. RESULTS

3.1 Preformulation Studies

TH was white, odourless and sticky powder while FLZ was almost white and odourless powder. The melting point of TH

and FLZ was found to be $205.66 \pm 1.15^\circ\text{C}$ and $139.66 \pm 0.57^\circ\text{C}$, respectively. The absorption maxima of TH and FLZ were found at 222 nm and 260 nm, respectively. The calibration curve of TH and FLZ in 0.1N HCl is shown in Fig. **1a** and **1b**, respectively.

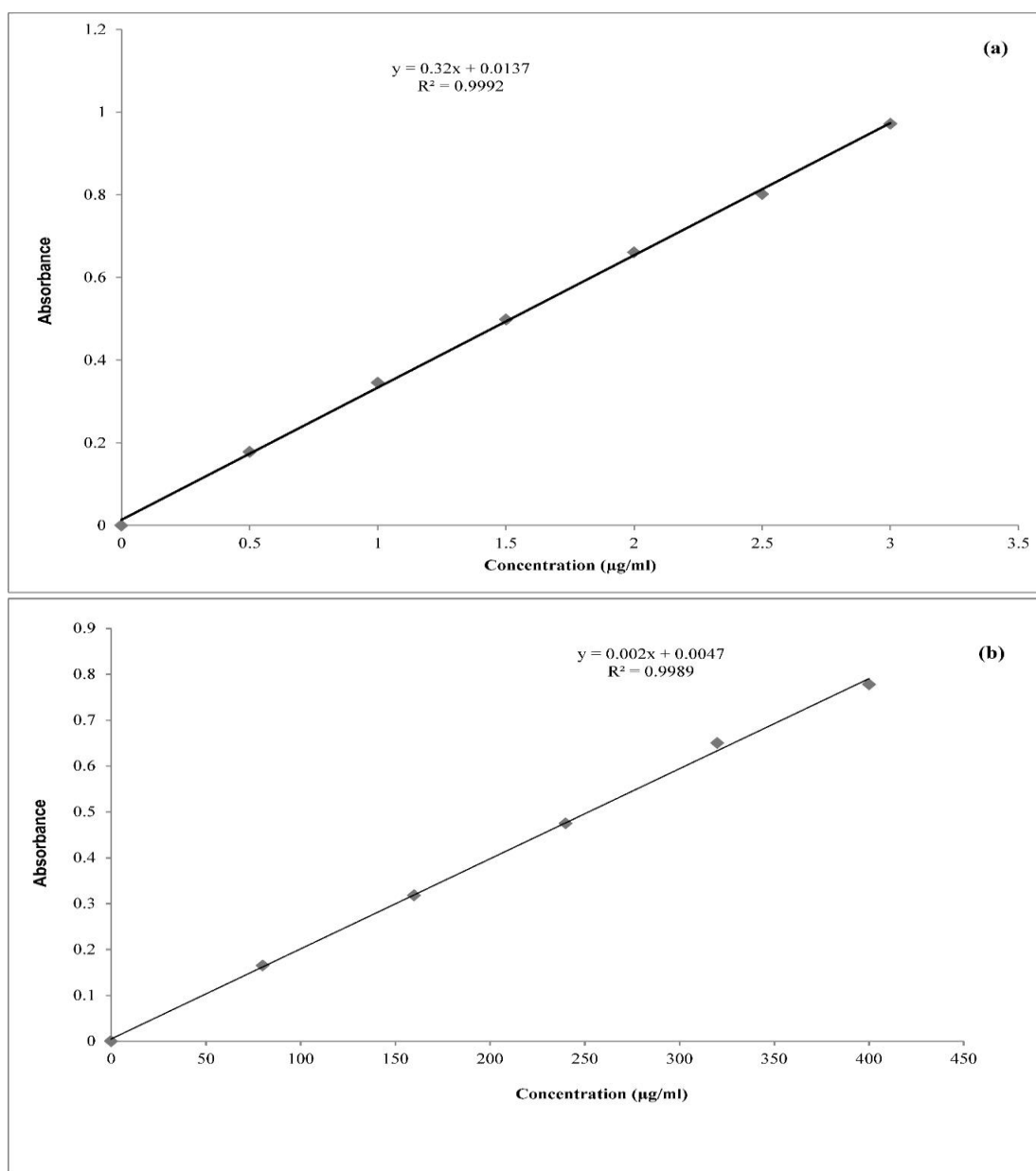


Fig. 1 Calibration curve (a) TH at 222 nm in 0.1N HCl (b) FLZ at 260 nm in 0.1N HCl

The FTIR spectrum of TH (Fig. **2a**) showed important peaks at 2869.54 cm^{-1} (-OH stretching), 2446.81 cm^{-1} (-SH stretching), 2224.98 cm^{-1} (-CN stretching), 1633.78 cm^{-1} (-C=O stretching), 1469.82 cm^{-1} (-C-H bending), 1072.47 cm^{-1} (-S=O stretching) and 777.35 cm^{-1} (-C-Cl stretching). The FTIR spectrum of FLZ (Fig. **2b**) showed important peaks at 3120.96 cm^{-1} (OH stretching), 1211.35 cm^{-1} (C-F stretching), 1621.24 cm^{-1} (-C=N stretching) and 2961.82 cm^{-1} (-C-H stretching). The FTIR spectrum of drugs in combination (Fig. **2c**) showed important peaks for TH at 2964.72 cm^{-1} (-OH stretching), 2446.81 cm^{-1} (-SH stretching), 2227.88 cm^{-1} (-CN stretching), 1621.24 cm^{-1} (-C=O stretching), 1465.96 cm^{-1} (-C-H bending), 1076.33 cm^{-1} (-S=O stretching) and

773.21 cm^{-1} (-C-Cl stretching). The important peaks for FLZ were observed at 3119.99 cm^{-1} (OH stretching), 1210.38 cm^{-1} (C-F stretching), 1621.24 cm^{-1} (-C=N stretching) and 2964.72 cm^{-1} (-C-H stretching). The FTIR spectrum of physical mixture of drugs with excipients used in formulation of FDC tablets (Fig. **2d**) showed important peaks for TH at 2970.50 cm^{-1} (-OH stretching), 2447.77 cm^{-1} (-SH stretching), 2225.95 cm^{-1} (-CN stretching), 1622.20 cm^{-1} (-C=O stretching), 1466.93 cm^{-1} (-C-H bending), 1078.07 cm^{-1} (-S=O stretching) and 776.38 cm^{-1} (-C-Cl stretching). The important peaks for FLZ were observed at 3119.99 cm^{-1} (OH stretching), 1208.46 cm^{-1} (C-F stretching), 1622.20 cm^{-1} (-C=N stretching) and 2970.50 cm^{-1} (-C-H stretching)¹⁵

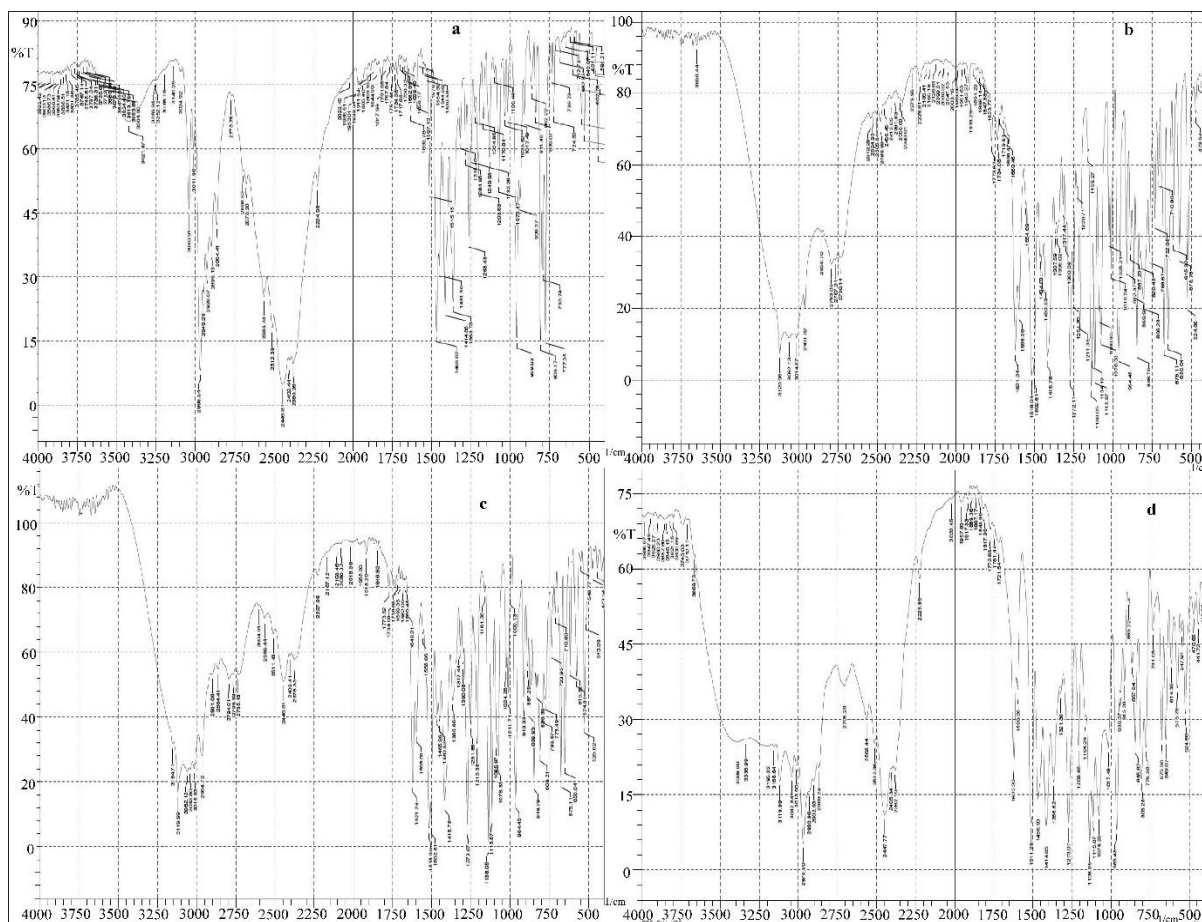


Fig 2. FTIR spectra (a) TH (b) FLZ (c) TH and FLZ physical mixture (d) Physical mixture of TH and FLZ with excipients in FDC tablets

3.2 Development and validation of simultaneous estimation method for TH and FLZ by UV-Visible spectrophotometer

The λ_{\max} of 3 $\mu\text{g/ml}$ solution of TH in 0.1N HCl was found to be 222 nm. The λ_{\max} of 400 $\mu\text{g/ml}$ solution of FLZ in 0.1N HCl was found to be 260 nm. In the overlay spectra (Fig. 3) three iso-absorptive points viz. 224 nm, 239 nm and 275 nm were reported and the iso-absorptive point 239 nm was selected for further analysis.

Linearity of TH was found in concentration range of 0.5-3.0 $\mu\text{g/ml}$ at 222 nm and 239 nm (Table 2, Fig. 4a and 4b).

Linearity of FLZ was found in the concentration range 160-400 $\mu\text{g/ml}$ at 222 nm and 239 nm (Table No. 2, Fig. 4c and 4d). The result of percent recovery study of proposed method is shown in Table 3. The percent recovery of TH at 222 nm and 239 nm was found in the range of $100.17 \pm 0.0032\%$ to $101.96 \pm 0.0026\%$ and $98.50 \pm 0.0010\%$ to $101.31 \pm 0.0006\%$, respectively. The percent recovery of FLZ at 222 nm and 239 nm was found in the range of $98.27 \pm 0.3125\%$ to $102.36 \pm 0.0210\%$ and $100.57 \pm 0.4318\%$ to $102.13 \pm 0.9174\%$, respectively.

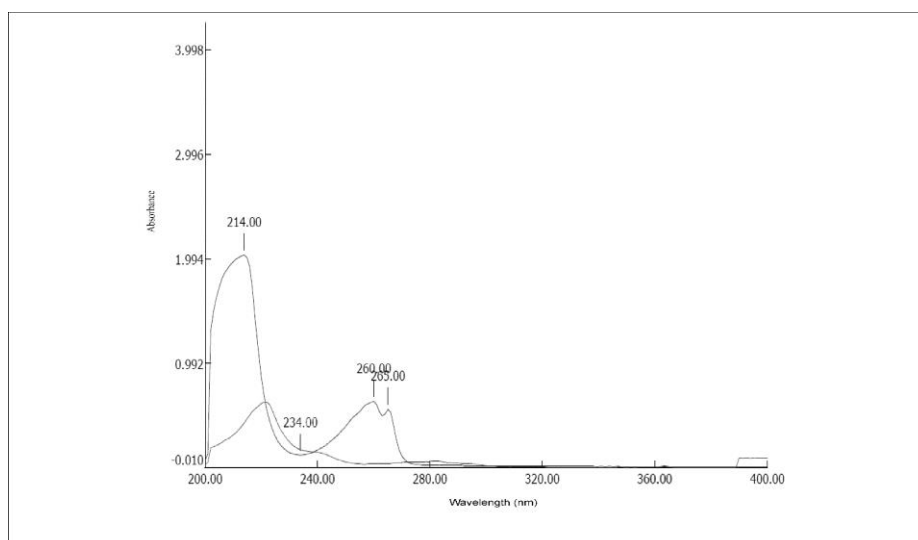


Fig. 3 Overlay spectrum of TH and FLZ in 0.1N HCl

Table No. 2 Linearity data of TH and FLZ at 222 nm and 239 nm in 0.1 N HCl

S.no	TH			FLZ		
	Concentration (µg/ml)	Absorbance at 222 nm	Absorbance at 239 nm	Concentration (µg/ml)	Absorbance at 222 nm	Absorbance at 239 nm
1	0.5	0.178 ± 0.0020	0.035 ± 0.0010	160	0.265 ± 0.0005	0.065 ± 0.0012
2	1.0	0.345 ± 0.0010	0.077 ± 0.0015	200	0.334 ± 0.0012	0.082 ± 0.0012
3	1.5	0.498 ± 0.0010	0.102 ± 0.0006	240	0.431 ± 0.0010	0.105 ± 0.0010
4	2.0	0.661 ± 0.0020	0.140 ± 0.0012	300	0.524 ± 0.0005	0.126 ± 0.0005
5	2.5	0.802 ± 0.0010	0.178 ± 0.0010	360	0.628 ± 0.0015	0.154 ± 0.0011
6	3.0	0.972 ± 0.0010	0.217 ± 0.0012	400	0.717 ± 0.0012	0.176 ± 0.0015

Mean ± SD; n=3

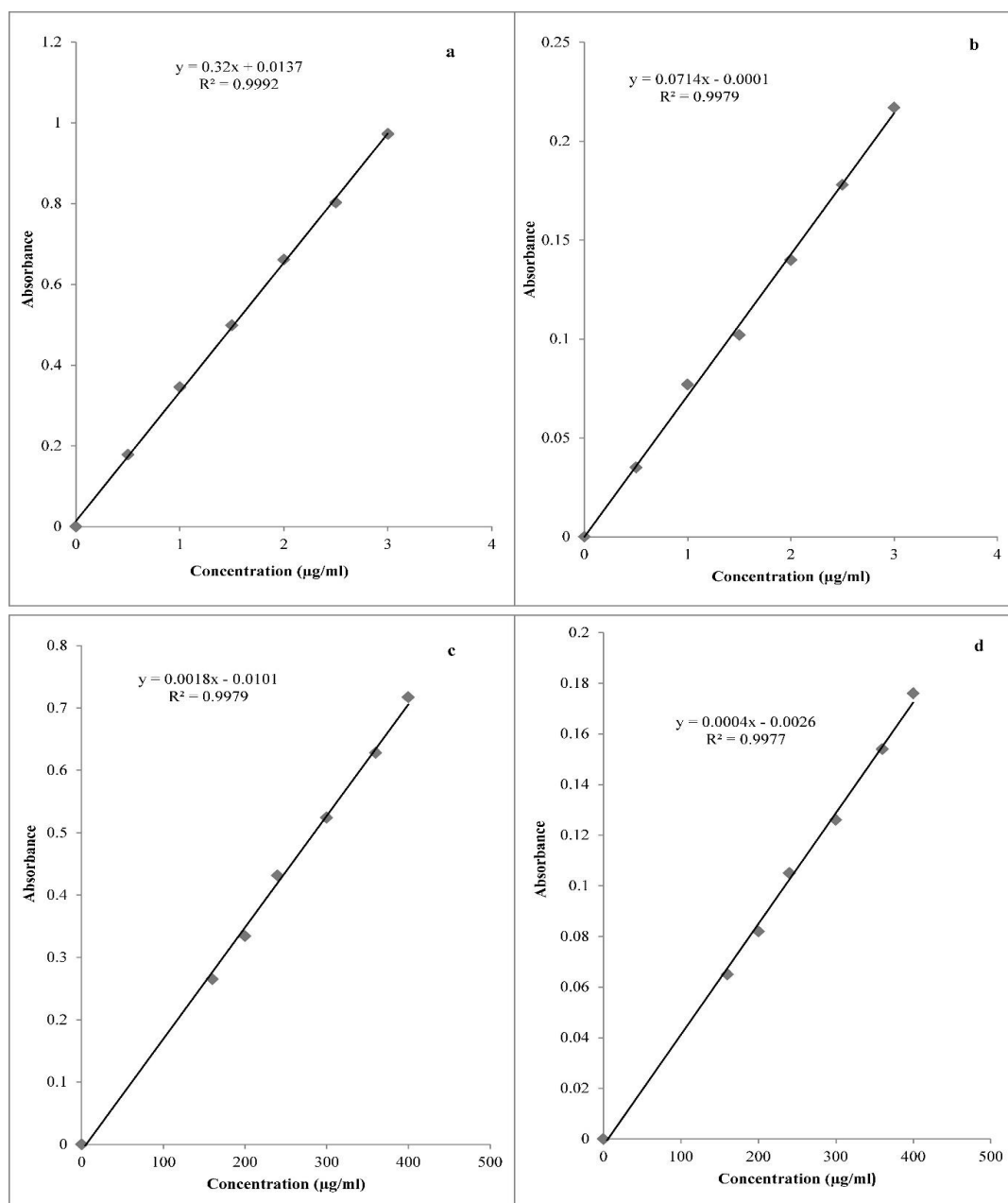


Fig. 4 Calibration curve (a) TH at 222 nm (b) TH at 239 nm (c) FLZ at 222 nm (d) FLZ at 239 nm

Table No. 3 Percent recovery of TH and FLZ at 222 nm and 239 nm in 0.1 N HCl

TH					FLZ				
Conc* (µg/ml)	222 nm		239 nm		Conc (µg/ml)	222 nm		239 nm	
	Conc found (µg/ml)	% R#	Conc found (µg/ml)	% R		Conc found (µg/ml)	% R	Conc found (µg/ml)	% R
2.0	2.03 ± 0.0057	101.40 ± 0.0360	1.97 ± 0.0026	98.50 ± 0.0010	240	245.66 ± 0.5946	102.36 ± 0.0210	242.86 ± 1.1335	101.19 ± 0.0316
2.5	2.5 ± 0.0011	100.17 ± 0.0032	2.51 ± 0.0069	100.31 ± 0.0006	300	302.55 ± 0.6755	100.85 ± 0.0315	301.71 ± 0.5861	100.57 ± 0.4318
3.0	3.06 ± 0.0208	101.96 ± 0.0026	2.99 ± 0.0549	99.90 ± 0.0017	360	353.77 ± 0.4229	98.27 ± 0.3125	367.66 ± 1.113	102.13 ± 0.9174

Mean ± SD; n=3, Conc*= Concentration, %R# = Percent recovery

The result of intermediate precision study is shown in Table No. 4. In determination of precision by repeatability study, percent relative standard deviation (%RSD) of TH at 222 nm and 239 nm was found to be 0.016 and 0.0193, respectively.

Similarly, % RSD of FLZ at 222 nm and 239 nm was found to be 0.481 and 0.0171, respectively. The results of reproducibility study are shown in Table No. 5.

Table No. 4 Intermediate precision of TH and FLZ at 222 nm and 239 nm in 0.1 N HCl

Drug	Concentration (µg/ml)	Absorbance at 222 nm				Absorbance at 239 nm			
		Inter-day	% RSD	Intra-day	% RSD	Inter-day	% RSD	Intra-day	% RSD
TH	2.0	0.641 ± 0.0031	0.483	0.646 ± 0.0017	0.216	0.146 ± 0.0012	0.822	0.144 ± 0.0010	0.694
	2.5	0.790 ± 0.0015	0.189	0.790 ± 0.0045	0.569	0.181 ± 0.0012	0.662	0.182 ± 0.0035	0.019
	3.0	0.917 ± 0.0031	0.338	0.911 ± 0.0064	0.702	0.216 ± 0.0006	0.277	0.217 ± 0.0010	0.555
FLZ	240	0.450 ± 0.0023	0.511	0.454 ± 0.0021	0.465	0.104 ± 0.0006	0.576	0.108 ± 0.0006	0.555
	300	0.562 ± 0.0015	0.267	0.556 ± 0.0010	0.180	0.129 ± 0.00058	0.387	0.127 ± 0.0012	0.945
	360	0.658 ± 0.0023	0.349	0.656 ± 0.0015	0.229	0.154 ± 0.0012	0.779	0.152 ± 0.0010	0.658

Mean ± SD; n=3

Table No. 5 Reproducibility of TH and FLZ at 222 nm and 239 nm in 0.1 N HCl

Drug	Concentration (µg/ml)	Absorbance at 222 nm				Absorbance at 239 nm			
		Analyst 1	Analyst 2	Calc. 't' value	Inference	Analyst 1	Analyst 2	Calc. 't' value	Inference
TH	2.0	0.643 ± 0.0015	0.646 ± 0.0030	0.8857	No significant difference	0.145 ± 0.038	0.149 ± 0.0035	0.4000	No significant difference
FLZ	300	0.566 ± 0.0020	0.565 ± 0.0040	0.8000	No significant difference	0.126 ± 0.0025	0.128 ± 0.0026	0.5066	No significant difference

Mean ± SD; n=3, *Calc.= Calculated

The LOD of TH at 222 nm and 239 nm was found to be 0.067 and 0.175 µg/ml, respectively. The LOD of FLZ at 222 nm and 239 nm was found to be 31.09 and 94.38 µg/ml, respectively. The LOQ of TH at 222 nm and 239 nm was

found to be 0.203 and 0.531 µg/ml, respectively. The LOD of FLZ at 222 nm and 239 nm was found to be 94.21 and 286.00 µg/ml, respectively.

3.3 Formulation and evaluation of FDC tablets

The results of evaluation of pre-compression parameters viz. mean granule size, tapped density, bulk density, Hausner's ratio, Carr's consolidation index, angle of repose, percent fines and percent moisture content of granules is shown in Table No. 6. The results of post-compression parameters viz.

appearance, average weight, thickness, hardness, percent friability, disintegration time and drug content is shown in Table No. 7. The result of *in vitro* drug release study of the formulations is shown in Table No. 8 and Fig. 5. The T_{80%} of TH and FLZ in F2 was found to be 11 and 10.5 minutes, respectively. The T_{80%} of TH and FLZ in F3 was found to be 12 and 11.5 minutes, respectively.

Table No. 6 Pre-compression parameter of granules formulated using HPC as binder

Formulation code	Average granule size (µm)	Loose bulk density (g/ml)	Tapped bulk density (g/ml)	Hausner's ratio	Carr's consolidation index (%)	Angle of repose (°)	Fines (%)	Moisture content (%)
F1	312.256 ± 0.600	0.574 ± 0.005	0.634 ± 0.008	1.233 ± 0.002	18.883 ± 0.142	26.61 ± 0.824	10.611 ± 0.067	1.584 ± 0.031
F2	320.447 ± 0.511	0.478 ± 0.004	0.588 ± 0.012	1.212 ± 0.004	17.523 ± 0.283	25.68 ± 0.813	9.381 ± 0.160	1.815 ± 0.035
F3	340.676 ± 0.956	0.468 ± 0.007	0.566 ± 0.011	1.208 ± 0.003	17.200 ± 0.235	23.79 ± 0.376	6.245 ± 0.068	1.642 ± 0.059
F4	366.133 ± 0.653	0.437 ± 0.003	0.520 ± 0.005	1.189 ± 0.004	15.960 ± 0.173	21.81 ± 0.306	5.137 ± 0.069	1.593 ± 0.049
F5	397.693 ± 1.223	0.407 ± 0.035	0.476 ± 0.008	1.169 ± 0.010	14.427 ± 0.837	20.28 ± 0.220	2.181 ± 0.035	1.948 ± 0.005

Mean ± SD; n=3

Table No. 7 Post compression parameters of FDC tablets formulated using HPC as binder

Formulation Code	Average weight (g)*	Thickness (mm)**	Hardness (kg/cm ²)**	Friability (%)#	Disintegration time (min)##	Drug content (%)#	
						TH	FLZ
F1	496.12 ± 0.0084	4.03 ± 0.020	4.67 ± 0.777	1.145 ± 0.039	-	-	-
F2	502.34 ± 0.0067	4.03 ± 0.011	6.72 ± 0.006	0.786 ± 0.069	07.21 ± 0.333	100.01 ± 0.318	102.94 ± 0.329
F3	503.01 ± 0.0070	4.02 ± 0.012	7.01 ± 0.012	0.674 ± 0.006	10.45 ± 0.436	97.61 ± 0.300	100.48 ± 0.323
F4	502.24 ± 0.0067	4.01 ± 0.009	8.23 ± 0.306	0.645 ± 0.051	18.38 ± 0.141	-	-
F5	504.21 ± 0.0057	3.99 ± 0.012	8.91 ± 0.200	0.556 ± 0.034	21.32 ± 0.156	-	-

Mean ± SD, * n=20, **n=10, # n=3, ##n=6

Table No. 8 *In vitro* drug release of FDC tablets formulated using HPC as binder

Time (min)	Cumulative drug release (%)	
	F2	F3
0	0	0
10	77.32 ± 2.597	75.30 ± 2.352
20	88.66 ± 3.001	86.41 ± 1.464
30	97.62 ± 2.421	91.32 ± 3.241
40	99.93 ± 1.276	99.62 ± 2.353
50	-	-
60	-	-

Mean ± SD, n=3

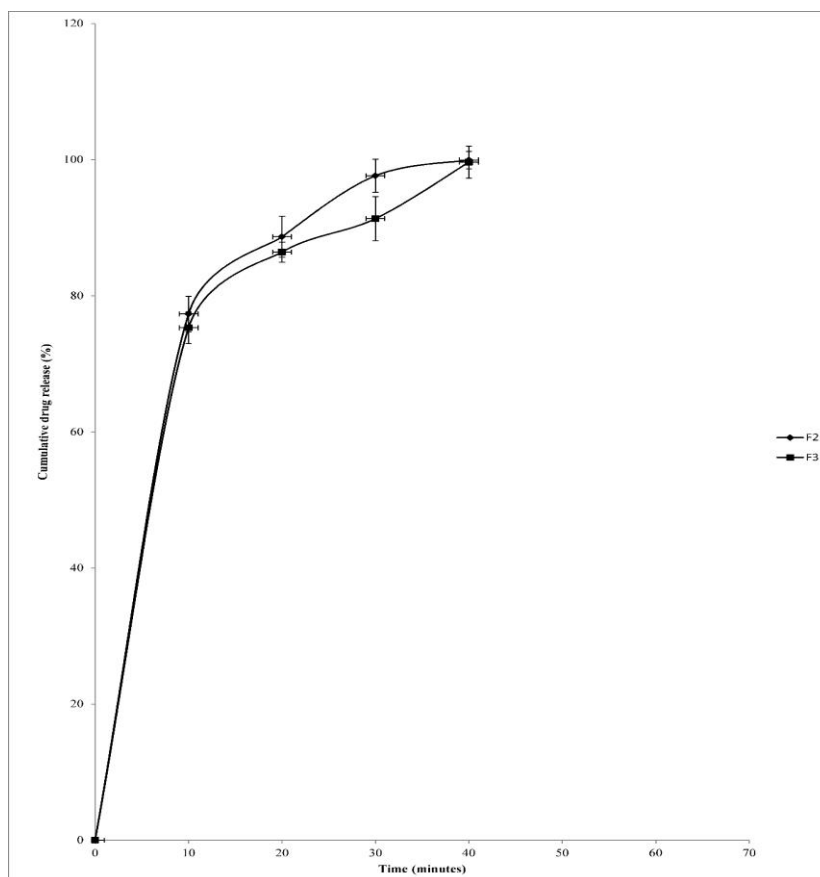


Fig. 5 Cumulative percent release of TH and FLZ from FDC tablets

4. DISCUSSION

4.1 Preformulation studies

The results of melting point determination of TH and FLZ indicated the purity of drug samples. The melting range of TH and FLZ reported in literature is 204-207°C and 138-140°C, respectively.

There was no major shift in the observed and standard peak positions of different functional groups in pure drug samples, indicating the purity of drug samples. Further, there was also no major change in the peak positions of different functional groups in FTIR spectrum of TH-FLZ physical mixture and TH-FLZ physical mixture with excipients as compared to the FTIR spectrum of pure drugs. Hence, The TH and FLZ together, and with the excipients used in tablet formulation were compatible.

4.2 Development and validation of simultaneous estimation method for TH and FLZ by UV-Visible spectrophotometer

The correlation coefficients of representative linear equations of TH and FLZ indicated very good linearity at 222 nm and 239 nm. Good accuracy of the developed method was pointed out by the good percent recovery. The %RSD values (< 2) in determination of intermediate precision and repeatability indicated good precision of proposed method [9]. There was no significant difference in the determination performed by two different analysts indicating good reproducibility of proposed method. The LOD and LOQ values of TH and FLZ on different wavelengths indicated good sensitivity of proposed method [9].

4.3 Evaluation of granules

The mean granule size increased with increased binder concentration. This might be because of better cohesion of solid mass due to improved wetting at higher binder concentration. The granules loose bulk density and tapped bulk density decreased with increased concentration of binder. This might be attributed to the increase average granule size with increased binder concentration.

Hausner's ratio is parameter that may be correlated to the flow property of granules. A value of angle of repose ≤ 1.25 indicates good flow and a value > 1.25 indicates poor flow. The Hausner's ratio decreased with increased binder concentrations. This might be because of increased mean granule size. All the formulations showed good flow property. Carr's consolidation index is related to the flow rate, cohesiveness and particle size of granules. Values below 15% represent good flow, 15 to 25% is fair/passable and values above 25% are indicative of poor flow. The F5 showed good whereas all the others formulation showed fair/passable flow property. Angle of repose also gives an idea of flow properties of granules. The angle of repose decreased with increased binder concentration, which indicated an improvement in flow. The increased granule size with increased binder concentrations might be the reason for this change. All the formulations were good flow properties¹⁶.

The percentage fines decreased with increased binder concentration which might be due to the better cohesion of solid mass at higher binder concentrations. The 1-2% of moisture content is necessary for compression of granules into tablets. If moisture content of granules is below 1% the tablets tends to break or laminate after compression¹⁷.

4.4 Evaluation of FDC tablets

The tablets were smooth, white, odorless and free from cracks or other surface irregularities. Average weight of tablets was found in between the range of 496.12 ± 0.0084 mg to 504.21 ± 0.0057 mg and thickness of tablets was found between the ranges of 3.99 ± 0.200 mm to 4.033 ± 0.200 mm, respectively (Table 7).

The hardness and percent friability of the tablets increased and decreased, respectively with increased binder concentration. The percent friability of F1 was more than 1%. Since, F1 failed in percent friability test, it was not considered for further evaluation. The disintegration time of the formulations increased with increased binder concentration. The disintegration time of formulation F2 and F3 was below 15 minutes and were considered for *in vitro* drug release study.

The drug content of F2 and F3 was found to be satisfactory. The rate of drug release decreased with increased binder concentration. The Indian pharmacopoeia states that uncoated conventional tablets should release 80% of drug within 30 min. Formulations F2 and F3 complies with the Indian Pharmacopoeia standard.

5. CONCLUSION

The developed FDC tablets can be a better alternative for treatment of *C. albicans* resistant to fluconazole. The TH and FLZ were compatible to each other and with the excipients used in the formulation of FDC tablet. The UV-visible spectrophotometric method developed for the simultaneous estimation of TH and FLZ was simple, accurate and sensitive. Further, the development of FDC tablets with other suitable pharmaceutical excipients and determination of synergistic effect *in vitro* or *in vivo* could be a future prospect for the developed FDC tablets.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise

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